Journal of Surgical Oncology 61:205–208 (1996)

Insulin-Like Growth Factor-II as a Prognostic Factor in Pulmonary Adenocarcinoma

IWAO TAKANAMI, MD, TETSUO IMAMUMA, MD, TOSHINORI HASHIZUME, MD, KEIICHI KIKUCHI, MD, YASUKI YAMAMOTO, MD, TATSUYA YAMAMOTO, MD, AND SUSUMU KODAIRA, MD

From the First Department of Surgery (I.T., Y.Y., T.Y., S.K.) and Department of Pathology (T.I.), Teikyo University School of Medicine, Tokyo, Japan; Department of Surgery, National Sanatorium, Kanagawa Hospital (T.H., K.K.), Kanagawa, Japan

We stained resected specimens from 117 patients with pulmonary adenocarcinoma for insulin-like growth factor-II (IGF-II) by the avidin-biotin-peroxidase (ABC) method and evaluated the usefulness of IGF-II as a prognostic factor. The patients were classified into the IGF-II (+) groups showing staining of 1% or more cancer cells (60 patients) and the IGF-II (−) groups showing staining of <1% (57 patients). The 5-year survival rate was 22% in the IGF-II (+) group and 54% in the IGF-II (−) group ($P < 0.01$). Our results suggest the usefulness of IGF-II stainability as a prognostic factor of pulmonary adenocarcinoma.

KEY WORDS: **insulin-like growth factor-II, pulmonary adenocarcinoma, prognostic factor, immunohistochemical staining, growth factor, proliferation**

INTRODUCTION

Insulin-like growth factor-II (IGF-II) is a polypeptide that consists of 67 amino acids and structurally resembles insulin and IGF-I [1]. Although the physiological roles of insulin and IGF-I have been clarified, the function of IGF-II is still unclear. IGF-II is markedly expressed in rat fetuses but decreases after birth [2]. In humans, IGF-II mRNA is increased in the kidney and liver of fetuses but is decreased in adults [3]. IGF-II is considered to be a growth factor needed for fetal growth, but its function in adults remained to be clarified. Increased expression of IGF-II mRNA or IGF-II has been reported in Wilm's tumor and pheochromocytoma [4,5]. IGF-II is also expressed in lung cancer cell lines and breast cancer cell lines [6,7]. In breast cancer cell lines, IGF-II has been reported to be mitogenic [8]. IGF-II is considered to be involved in proliferation by the autocrine and/or paracrine mechanisms [7]. Some growth factors involved in proliferation by these mechanisms can be prognostic factors of cancer [9]. However, there are no reports that evaluated the usefulness of IGF-II as a prognostic factor.

Among the histological types of lung cancer, the incidence of adenocarcinoma is the highest in Japan [10] and has recently been increasing in the world [11]. The behavior of pulmonary adenocarcinoma is not adequately understood, and the results of its surgical and medical treatment are not satisfactory. To determine whether IGF-II can be a prognostic factor, we stained resected specimens of pulmonary adenocarcinoma for IGF-II by an immunohistological method and evaluated the relationship between IGF-II expression and outcomes.

MATERIALS AND METHODS

Sources of Tissues

Tissue specimens were obtained from 117 patients with pulmonary adenocarcinoma who underwent resection at our department between 1981 and 1989. Patients who died within 1 month after operation or who underwent exploratory thoracotomy were excluded. Neither cases with past history of another cancer nor cases found to have another cancer at closer examination before operation for lung cancer were included in our series.

Staging of lung cancer was based on surgical and pathological findings according to the TNM classification proposed by the UICC in 1987. Stage I was observed in 50 patients, stage II in 8, stage III A in 35, stage III B in 2, and stage IV in 22. The patient group included 69 males

Accepted for publication September 20, 1995.

Address reprint requests to Dr. Iwao Takanami, The First Department of Surgery, Teikyo University of Medicine, 11-1, Kaga 2-Chome, Itabashi-Ku, Tokyo, 173, Japan.

and 48 females. Their age ranged from 28 to 81 years (mean, 61 yr). The degree of histological differentiation was classified according to the WHO classification [12]. Since the degree of differentiation of adenocarcinoma sometimes differs among areas of the tumor, the most predominant degree of differentiation in each tumor was adopted. The well-differentiated type was observed in 48 patients, moderately differentiated type in 44, and poorly differentiated type in 25. Surgery was performed in patients in whom radical operation was considered to be possible by lobectomy or pneumonectomy with dissection of complete hilar and mediastinal lymph nodes. All patients were followed up for 5–12 years, and their outcomes were confirmed.

Immunohistochemistry

Resected specimens were fixed in formalin, embedded in paraffin, cut into 3-μm sections, and stained with hematoxylin-eosin and by an immunohistological method for IGF-II. As anti-IGF-II monoclonal antibody, mouse antirat IGF-II monoclonal antibody (Upstate Biotechnology, Lake Placid, NY) was used. The cross-reactivity of this antibody was 100% for human IGF-II, 100% for rat IGF-II, and 10% for human IGF-I. These specifications were confirmed by neutralization and Western blot analysis [13]. To avoid cross reaction with IGF-I, the antibody was absorbed by 1 μg of IGF-I (Kaigen Co. Stockholm, Sweden).

IGF-II immunohistochemical staining was performed by the ABC method [14] using a Vestatin Kit (Vector Co., Burmingame, CA). After deparaffinization, endogenous peroxidase was blocked. The specimens were washed with phosphate-buffered saline (PBS) reacted with 10% normal rabbit serum (Vector) and reacted with mouse antirat IGF-II monoclonal antibody diluted 1/100 as the first antibody at 4°C overnight and with biotinylated rabbit antimouse serum (Vector) as the second antibody at room temperature for 1 hour. After reaction with drops of the avidin-biotin-peroxidase complex at room temperature for 60 minutes, color was developed with diaminobenzine. Nuclear staining was performed with methyl green. Human tonsils were used as control tissue.

Analysis

Assessment of IGF-II immunohistochemical staining of pulmonary adenocarcinoma was performed by at least two independent observers who did not know the stage, histological type, or patient history. The extent of immunoreactivity was grouped into four degrees as follows: −, no cancer cell staining or immunoreactivity staining of <1% of tumor cells; +, immunoreactivity staining of 1 ~ 24% of tumor cells; ++, moderate immunoreactivity staining of 25 ~ 74% of the tumor cells; +++, intense staining of >75% of the tumor cells.

The possible correlation between the results of IGF-II staining and sex, TNM stage, degree of histologic differentiation, or curative resection was analyzed by the Chi-square test. Survival rates were calculated by the Kaplan-Meier method and compared by the log rank test. Multivariate analysis was done using Cox's proportional hazards regression model [15]. Computer calculation was performed using the SAS/STAT statistical package (Statistical Analysis System Institute, Cary, NC) and an NEC system PC 9801 RA computer. Significance was set at $P < 0.05$.

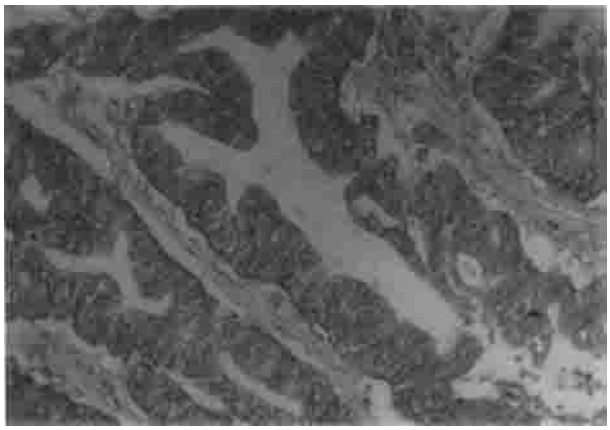

Fig. 1. IGF-II Positive staining in well-differentiated adenocarcinoma (×66). Because of the marked diffuse cytoplasmic staining of the cancer cells, this patient was assigned to the IGF-II (+) group.

RESULTS

Immunohistochemical staining showed marked IGF-II expression in the cytoplasm of cancer cells (Fig. 1). The cancer stroma was stained in a few patients who also showed marked staining in cancer cells. The normal alveoli, normal bronchial epithelium, and the smooth muscle were slightly stained.

Of the 117 patients, 57 (49%) were classified as −, 21 (18%) as +, 19 (16%) as ++, and 20 (17%) as +++. Assessed data included factors of TNM stage, pathologic grade of differentiation, and according to the extent of IGF-II. There was no statistically significant difference among the four degrees of the immunoreactivity of IGF-II. The 5-year survival rates and patients with −, +, ++, and +++ were 54%, 24%, 22% and 20%, respectively. The extent of −, showing staining of <1% of tumor cell, was defined as IGF-II (−) and that of +, ++, +++, showing staining of 1% or more cancer cells, was defined as IGF-II (+). Evaluation of the possible correlation between IGF-II stainability and sex, T (tumor), N (node), M (metastasis), stage, degree of histological differentiation, or curative resection revealed no significant differences (Table I).

The survival curves in the IGF-II (+) and IGF-II (−) groups are shown in Figure 2. The 5-year survival rate

TABLE I. IGF-II Expression in Pulmonary Adenocarcinoma

Variables	Negative (n = 57)	Positive (n = 60)	*P* value*
Sex			
Males	34	35	
Females	23	25	NS[b]
P stage[a]			
I	31	19	
II	5	3	
IIIA	13	22	
IIIB	1	1	
IV	7	15	NS
T factor[a]			
T 1	27	20	
T 2	26	33	
T 3	4	7	NS
N factor[a]			
N 0	33	28	
N 1	5	3	
N 2	17	27	
N 3	2	2	NS
M factor[a]			
M 0	50	45	
M 1	7	15	NS
Differentiation			
Well	22	26	
Moderate	21	23	
Poor	14	11	NS
Curability			
Curative	49	44	
Noncurative	8	16	NS

* χ^2 test.
[a] International TNM staging system for lung cancer by Mountain [19].
[b] NS, not significant.

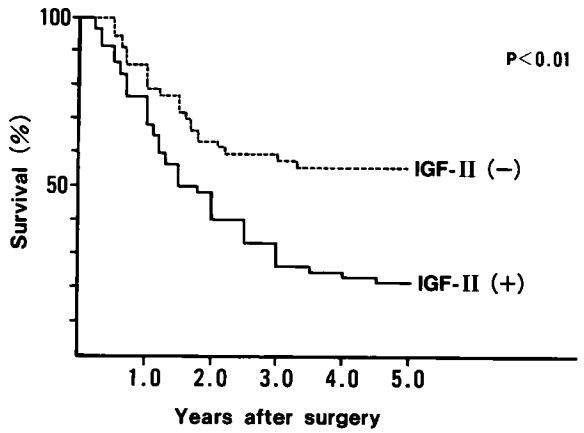

Fig. 2. Survival curves of patients with pulmonary adenocarcinoma according to the classification by IGF-II. A significant difference is indicated between the two groups ($P < 0.01$).

was 54% in the IGF-II (−) group and 22% in the IGF-II (+) group with a significant difference ($P = 0.004$). To determine whether IGF-II is an independent prognostic factor, data in 93 patients who underwent potentially curative resection were evaluated by multivariate analysis (Table II). IGF-II as well as *P*-stage (*P*-stage I, II, III a) was an independent prognostic factor (IGF-II, $P = 0.0114$; *P*-stage, $P = 0.0001$).

TABLE II. Multivariate Analysis of Cox's Proportional Hazards Model in 93 Curatively Resected Patients With Pulmonary Adenocarcinoma

	Multivariate analysis			
Variables	Parameter estimate	Standard error	χ^2	*P* value
IGF-II	0.761389	0.30091	6.904253	0.0114
P stage[a]	0.875029	0.16651	27.61620	0.0001

[a] International TNM staging system for lung cancer by Mountain [19].

DISCUSSION

The marked expression of IGF-II and mRNA of IGF-II in tumors compared with the normal tissue has suggested promotion of human tumor growth by IGF-II [4]. This promotion is considered to be mediated by both IGF-I receptors and IGF-II/mannose 6 phosphate receptors [16]. In breast cancer, IGF-I receptors play an important role in proliferation and progression, and the prognosis is poor in patients positive for IGF-I receptors, suggesting the usefulness of IGF-I as a prognostic factor [17]. The presence of IGF-I and IGF-II receptors were reported in lung cancer [18]. However, there are no studies that evaluate the relationship between IGF-II and the prognosis of lung cancer.

In this study, we evaluated the relationship between IGF-II expression and prognosis using anti-IGF-II monoclonal antibody in pulmonary adenocarcinoma. Immunohistochemical staining in 117 patients with pulmonary adenocarcinoma showed IGF-II expression in 51%. The action mechanism of IGF-II is considered to be autocrine and/or paracrine tumor cell proliferation. In this study, IGF-II was markedly expressed in cancer cells but was also slightly present in fibroblasts in the cancer stroma. IGF-II was slightly expressed in the normal bronchial epithelium, and it was not merely nonspecific staining. IGF-II may be involved in autocrine and paracrine stimulation in epithelial proliferation and repair, without an associated malignant transformation. Comparison of IGF-II staining with TNM stage, degree of histological differentiation, and curative resection did not identify any significant correlation. IGF-II +++ staining was supposed to confer a worse prognosis. In fact, the 5-year survival rates of patients with +++ IGF-II staining was 20%, which was a worse prognosis. But there were no statistically significant differences in survival rates among +,++, and +++ IGF-II. The outcome significantly differed between the IGF-II (+) and (−) groups ($P = 0.004$). Multivariate analysis also suggested that IGF-II can be

a prognostic factor ($P = 0.0114$). These data suggest that IGF-II may contribute to progression of pulmonary adenocarcinoma. IGF-II stainability can serve as a prognostic factor for patients undergoing surgery of pulmonary adenocarcinoma. To evaluate the role of IGF-II in pulmonary adenocarcinoma, studies are needed on its association with the expression of IGF-II receptors. Although antibodies to IGF-II receptors were tried to stain specimens embedded in paraffin, they did not respond. Although further studies are needed on IGF-II and related receptors to evaluate the role of IGF-II in lung cancer, IGF-II stainability was related to poor prognosis.

CONCLUSION

The expression of IGF-II was an independent prognostic factor for pulmonary adenocarcinoma. The IGF-II positive group had a poorer prognosis than the negative group. These data suggest that IGF-II may contribute to progression of pulmonary adenocarcinoma. A study determining the relationship between the poor prognosis of pulmonary adenocarcinoma and positive immunoreactivity staining of IGF-II should aid in the diagnosis and treatment of these patients.

REFERENCES

1. Rindeknecht E, Humble RE: The amino acid sequence of human insulin-like growth factor and its structural homology with proinsulin. J Biol Chem 253:2769–2776, 1978.
2. Rotwein P, Pollock KM, Watson M, Milbrandt JD: Insulin-like growth factor gene expression during rat embryonic development. Endocrinology 121:2141–2144, 1987.
3. Scott J, Cowell J, Robertson ME, et al.: Insulin-like growth factor-II gene expression in Wilms' tumor and embryonic tissues. Nature 317:260–262, 1985.
4. Reeve AE, Eccles MR, Wilking RJ, et al.: Expression of insulin-like growth factor II transcripts in Wilms' tumor. Nature 317:258–262, 1985.
5. Haselbacher GK, Irminger JC, Zaph J, et al.: Insulin-like factor in human adrenal pheochromocytomas and Wilms tumors: Expression at the mRNA and protein level. Proc Natl Acad Sci USA 84:1104–1106, 1987.
6. Haveman K, Rotsch M, Schoneberger HJ, et al.: Growth regulation by insulin-like growth factor in lung cancer. J Steroid Biochem Mol Biol 37:877–882, 1990.
7. Osborne CK, Coronado EB, Kitten LJ, et al.: Insulin-like growth factor-II (IGF-II): a potential autocrine/paracrine growth factor for human breast cancer via the IGF-I receptor. Mol Endocrinol 3:1701–1709, 1989.
8. Myal Y, Shiu RPC, Bhaumick B, Bala M: Receptor binding growth-promoting activity of insulin-like growth factors in human breast cancer cells (T-47D) in culture. Cancer Res 44:5486–5490, 1984.
9. Tateishi M, Ishida T, Mitsudomi T, et al.: Immunohistochemical evidence of autocrine growth factors in adenocarcinoma of the human lung. Cancer Res 50:7077–7080, 1990.
10. Nitani H: Registration report of lung cancer in Japan (III), classification of saging and lung cancer according to TNM Case Summary from 1967–1969, 1975.
11. Valaitis J, Warren S, Gamble D: Increase incidence of adenocarcinoma of the lung. Cancer 47:1042–1047, 1981.
12. World Health Organization histological typing of lung tumors (WHO). Am J Clin Pathol 77:123–136, 1982.
13. Tanaka H, Asami O, Hayami T, et al.: Identification of a family of insulin-like growth factor II secreted by cultured rat epithelial-like cell line 18, 54–3F: Application of monoclonal antibody. Endocrinology 24:870–877, 1989.
14. Hsu SM, Raine L, Fanger H: Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: A comparison between ABC and unlabeled antibody (PAP) procedure. J Histochem Cytochem 29:577–580, 1981.
15. Cox DR: Regression models and life tables. J Stat Soc B34:187–220, 1972.
16. Morgan DO, Edman JC, Standing DN, et al.: Insulin-like growth factor II receptor as a multifunction binding protein. Nature 329:301–307, 1987.
17. Railo MJ, Smitten KV, Pekonen F: The prognostic value of insulin-like growth factor-I in breast cancer patients: Results of a follow-up study on 126 patients. Eur J Cancer 30A:309–311, 1994.
18. Kaiser U, Schardt C, Ebandscheidt D, et al.: Expression of insulin-like growth factor receptors I and II in normal human lung and in lung cancer. J Cancer Res Clin Oncol 119:665–668, 1993.
19. Mountain CF: A new international staging system for lung cancer. Chest 89:225–232, 1986.